DOWN REGULATION OF INSULIN RECEPTORS

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Although this paper specifically deals with down regulation of insulin receptors, it is well to recognize that down regulation is a widespread phenomenon exhibited by many hormone receptors. Down regulation refers to the reduction in cellular receptors for a specific hormone when the concentration of that hormone is increased. Similarly, up regulation, which is a less common phenomenon, denotes an increase in specific receptors when the concentration of its hormone is increased.

The ubiquity of hormone receptor regulation by homologous hormone can be seen in Table I. The physiologic role of down and up regulation is somewhat speculative. Up regulation may be a method by which the hormone's effect may be amplified. Hormone receptors demonstrating up regulation may be present in too small a quantity to initiate a biologic effect until their concentration is increased by the homologous hormone. For a hormone such as prolactin which fluctuates widely during the day and responds to perturbations in energy fuels, a necessity for up regulation before the full effect of the hormone is exhibited may protect the animal from undesirable fluctuation in hormone activity (lactation). Down regulation of hormone receptors is much easier to explain teleologically since a decrease in receptors would protect the animal from hormone excesses. A homeostatic protective effect of down regulation may be reflected by the insulin and catecholamine insensitivity exhibited by patients with islet cell tumors[2] and pheochromocytomas,[3] respectively. Perhaps even the insulin resistance observed after hypoglycemia in the diabetic (the Somogyi phenomenon) may be explained in part by decreased insulin receptors secondary to iatrogenic hyperinsulinism. Since insulin resistance persists even after the counter-regulatory hormone levels return to normal, some additional source of resistance must be sought. The remainder of the paper will treat specifically of down regulation of insulin receptors.

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Down regulation of insulin receptors may be invoked to explain the abnormalities in insulin receptor concentration seen in most physiologic and pathologic conditions to date in which receptor aberrations have been reported. Table II lists these conditions. In all of the conditions in which decreased insulin receptors have been demonstrated, hyperinsulinism and some degree of insulin resistance exist. Caution must be exercised not to attribute the insulin resistance in these conditions solely to decreased numbers of insulin receptors since in at least two of the conditions, obesity[4] and steroid excess,[5] a post-receptor intracellular block in glucose metabolism has been demonstrated which is probably responsible for the insulin resistance. The only pathologic condition in which a decrease in insulin receptors has been definitely shown to be the cause of insulin resistance and hyperinsulinism is the rare diabetic syndrome in patients, usually with other manifestations of autoimmune disease, who exhibit auto-antibodies to the insulin receptor.[6] The conditions on the right hand side of Table II are those shown to be associated with increased insulin receptor number. These conditions are characterized by low circulating insulin levels, and as a corollary of down regulation would be expected to exhibit increased insulin receptors.

In the two conditions most commonly associated with decreased insulin

TABLE I

Regulation of Hormone Receptor Concentration by Homologous Hormone

Down Regulation	Up Regulation
Insulin	Prolactin
Growth Hormone	FSH
Catecholamines	LH
Angiotensin	
Estrogen	
Progesterone	
TRH	

This table was modified and adapted from an article published by Lesniak and Roth,[1] with permission of the authors. References for the observations may be found in the original article.[1]

TABLE II

Conditions Associated with Abnormal Numbers of Insulin Receptors

Decreased Receptors	Increased Receptors
Obesity	Fasting
Hyperinsulinemic Diabetes	Exercise
Cushing's Syndrome*	Insulinoprivic diabetes*
Acromegaly	
Renal Disease*	
Congenital Lipodystrophy	

* Demonstrated in animal studies only.

receptors—obesity and diabetes—an inverse relationship between insulin concentration and insulin receptor number has been demonstrated.[4, 7] In the case of obesity, weight loss causes a return of insulin receptor number to normal along with a decrease in the hyperinsulinism.[7] In insulin-independent diabetes, chronic treatment with oral sulfonylurea agents which causes a paradoxical reduction of plasma insulin concentration, normalizes the insulin receptor number.[8] These studies in obesity and diabetes offer only circumstantial evidence for down regulation of insulin receptors. They do not clearly establish whether the reduced insulin receptor number is the cause or effect of hyperinsulinism in these conditions.

To answer the above question, we designed an experiment in which insulin levels would be suppressed without a change in weight in obese subjects. Such a manipulation allowed us to assess the role of hyperinsulinism in the decreased receptor population observed in obesity. Diazoxide (5 mg/kg/day), a potent suppressor of insulin release, was administered to 10 obese non-diabetic subjects and receptor quantitation was performed on peripheral mononuclear cells before and after drug administration. Basal and glucose stimulated insulin release was suppressed in all but one subject. An increase in maximal insulin binding to the mononuclear cells was observed in nine of the ten subjects (Table III), although further analysis of the binding curves revealed a clear increase in receptor number (low or high affinity binding sites) in only seven of the ten subjects. We interpret this study as indicating that the decreased insulin receptor number of obesity is a result, not a cause of hyperinsulinism. The hyperinsulinism in obesity may be attributed to insulin resistance due to a post-receptor block as suggested by others.[4]

TABLE III

Effect of Diazoxide and Insulin Suppression on Insulin Binding by Peripheral Mononuclear Cells in Obesity

Patient	% ^{125}I-Insulin Binding/20 × 10^6 Mononuclear Cells	
	Before Diazoxide	After Diazoxide
AA	4.7	6.0
EB	2.5	5.0
SM	4.8	5.9
KP	2.6	6.3
MM	2.2	5.0
JC	3.1	5.8
HN	4.0	8.9
DB	7.0	7.0
PC	4.8	4.9
EH	2.7	3.0
Mean ± SEM ($p < .02$)	3.84 ± .48	5.78 ± .49

The clinical investigations just described represent indirect evidence for down regulation. Direct evidence for down regulation was first reported from the NIH group who used cultured IM-9 lymphocytes to demonstrate the effect of enrichment of media with insulin on cellular insulin receptors.[9] In these studies, the lowest concentration of insulin which consistently demonstrated a suppressive effect on insulin receptor number was 10^{-8} M, a supraphysiologic concentration. We performed similar studies in a cell type of greater importance in glucose homeostasis, the hepatocyte, and were able to demonstrate down regulation at a more physiologic insulin concentration (10^{-9} M).[10] For our studies, we employed the adult rat hepatocyte in monolayer culture. These cells are viable for many days and carry out numerous functions characteristic of the liver *in vivo*. Incorporation of insulin at 10^{-9} M and 10^{-8} M concentrations for 16 hours prior to harvesting the cells resulted in significant down regulation of insulin receptors as demonstrated by the Scatchard plots shown in Figure 1.

The mechanism by which down regulation occurs is not understood at present. Studies by Dr. J. Roth and co-workers with cycloheximide have suggested that down regulation of insulin receptors[11] as well as growth hormone receptors[1] is due to increased degradation, rather than decreased synthesis of receptors. In fact, Cuatrecasas has proposed that down

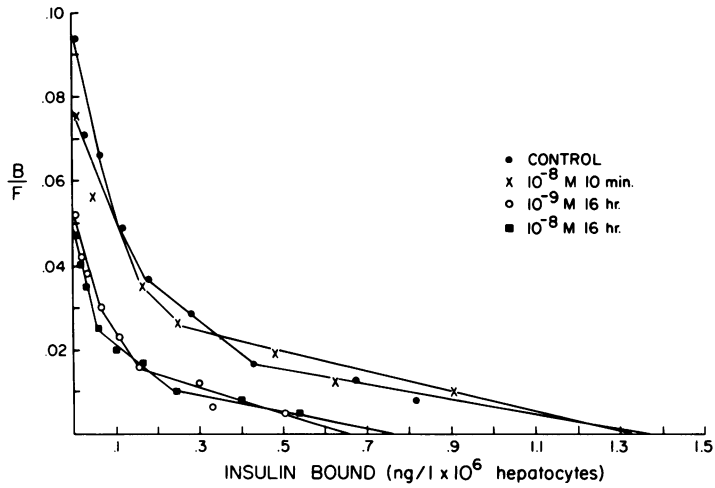

FIG. 1. Effect of chronic insulin exposure (16 h) on insulin receptors on cultured hepatocytes. The medium was changed every 4 h and 2×10^{-8} and 4×10^{-9} M insulin were used. The insulin additions were calculated to compensate for degradation over the 4-h periods and to maintain a concentration of at least 10^{-9} or 10^{-8} M. The control cells incubated in the absence of insulin for an additional 16 h were washed in the same manner as were the experimental cells.

regulation of insulin receptors may simply be a manifestation of the proteolytic effect of insulin.[12] It is unlikely, however, that down regulation of insulin receptors by insulin at a physiological concentration can be attributed to insulin's proteolytic activity.[13]

In an attempt to shed further light on the mechanism by which down regulation of insulin receptors occurs, we examined the effect of other agents with insulin-like activity on insulin receptor concentrations. For these experiments, we employed IM-9 lymphoblastoid cells in culture. Table IV lists some of the substances tested. Of these, the only substance with insulin-like activity which clearly decreased insulin receptor number without affecting cell viability was the plant lectin, concanavalin A. Polyamines, in the presence of albumin, and hydrogen peroxide may also decrease insulin receptors, but we were unable to demonstrate decreased receptors at concentrations which did not affect cell viability.

Down regulation of insulin receptors by Con A is shown in Figure 2. The concentrations of Con A employed in these studies (5 and 10 μg/ml) neither cause cell agglutination, nor do they compete with insulin for binding to the cells. Down regulation of insulin receptors by Con A is prevented by the simultaneous addition of alphamethylmannoside, a sugar known to inhibit other actions of Con A by preventing its binding to cell surface glycosyl residues. Exactly how Con A produces its insulin-like effects is unclear. Although the insulin-like effects of Con A (down regulation of receptors and enhanced glucose oxidation) occur at concentrations which do not inhibit insulin binding to the receptor, Con A may bind to a portion of the receptor not necessary for insulin binding, since Cuatrecasas has shown Con A binding to solubilized insulin receptors.[14] Whether Con A binds to the specific insulin receptor or an adjacent site influencing the insulin receptor is not clear at this time. However, it is apparent that the insulin-like activity of Con A and its capacity to down regulate insulin receptor number are closely related. We have performed additional studies with succinyl Con A, a divalent derivative which

TABLE IV

Down Regulation by Substances with Insulin-Like Activity

Concanavalin-A	Positive
Polyamines	Positive, but cell viability decreased
H_2O_2	Positive, but cell viability decreased
Vitamin K_5	Positive, but cell viability decreased
Phospholipase C	Negative
B. Subtilis Protease	Negative
S. Greisens Protease	Negative
Somatomedin	Negative
Growth Hormone Fragments	Negative
Imidazol	Negative

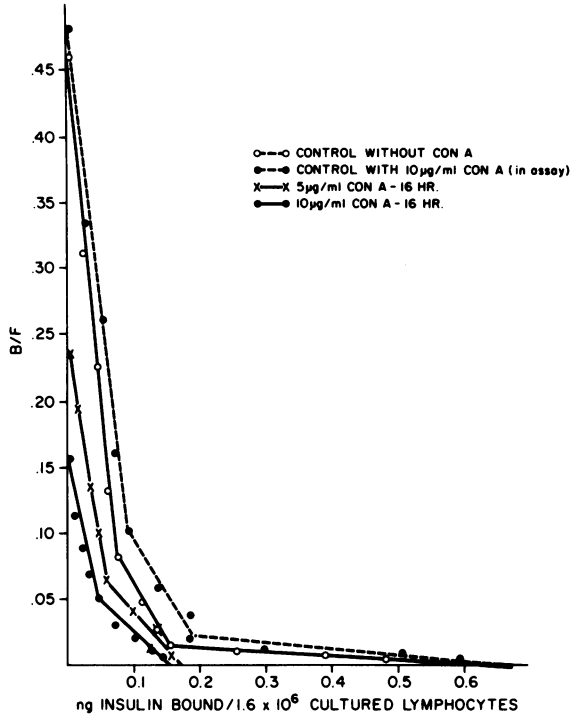

FIG. 2. Down regulation of insulin receptors by concanavalin A. IM-9 lymphoblastoid cells were incubated with Con A for 16 h before receptor quantitation. Con A, in the concentrations used for down regulation, did not inhibit insulin binding to the cells acutely when added to the assay.

exhibits only 10% of the insulin biologic activity of natural tetravalent Con A. This substituted compound was also only ten percent as effective in down regulating insulin receptors.

The correlation between down regulation and the other biologic effects of insulin raised the question whether down regulation of insulin receptors might be a result of one of the metabolic actions of insulin on the cell. To explore this possibility, we examined the effect of some metabolic inhibitors on down regulation of insulin receptors by insulin. Mannoheptulose and 2-deoxyglucose, which block different steps of glycolysis failed to block down regulation as did also cytochalasin-B, a compound which inhibits glucose transport. Theophylline, a phosphodiesterase inhibitor which theoretically might inhibit insulin's suppressive effect on intracellular cyclic AMP accumulation was tested and also failed to block down regulation. Cyclic AMP was a particularly attractive hypothetical mediator of receptor concentration since the cyclic nucleotide has been demonstrated to increase insulin receptors.[15] In this regard, imidazol, an agent

which depressed cyclic AMP levels was unable to mimic the down regulatory activity of insulin. The preceding experiments do not absolutely disprove a post-receptor event as being responsible for down regulation. However, our bias is that down regulation occurs as a result of hormone-receptor interaction and that this interaction may be reflected by insulin biological activity, but that the post-receptor activity plays little role in down regulation.

We have tried to present an overview of down regulation of insulin receptors drawing from the work of others as well as ourselves. The mechanism by which down regulation occurs remains obscure, but its importance in physiologic and pathologic states is obvious. Such a regulatory process adds a new dimension to our understanding of the fine tuning which the body is capable of in order to maintain homeostasis.

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DISCUSSION

DR. BRANSOME (Augusta): "What you have implied by the phrase 'down regulation' is intriguing in that similar reports have been forthcoming in a variety of experiments with many hormones. Have you had a chance to look at the kinetics of down regulation in your systems and the IM-9 systems? I am mindful of the work published from Roth's group some years ago, with similar cellular systems in which down regulation took some time to occur, almost 16 hours. Have you repeated that sort of observation, or can the decrease of insulin receptors be demonstrated more rapidly? If it can, 'down regulation' would seem a little closer to what we can observe in patients. A second, related question should allow you to muse and speculate a bit: what about repletion of those receptors? How fast can that occur and under what conditions?"

DR. BLACKARD (Richmond): "We have looked at this in a rather sketchy fashion, but the time required for down regulation is dose-dependent. Cuatrecasas has shown down regulation in the adipocyte in a very short period of time, but since he was unable to culture the adipocytes he had to put the cells in a very high concentration of insulin to demonstrate the effect. I think the concentrations he used were 10^{-4} M to 10^{-5} M which are clearly supraphysiologic. So the effects can be demonstrated in a shorter period of time with very high concentrations of insulin. Actually what Cuatrecasas has said is that he thought that in his experiments the loss of receptors was due to the proteolytic action of insulin and that may be true in such high concentrations. But in the physiologic concentrations of insulin we use (10^{-8} M and 10^{-9} M), insulin does not have a proteolytic effect. The time required for down regulation with what I would consider physiologic concentrations is something more than eight hours—we looked at eight hours and we looked at 24 hours—we could not demonstrate the effect at eight hours, but could at 24 hours at an insulin concentration of 10^{-8} M. We did not try higher concentrations. With con-A, the effect occurs somewhat quicker, but again that may be dose-dependent, and it occurred at about two-and-a-half hours. Your second question: the time required for repletion of the receptors—we again had examined this only in a very sketchy manner, and our points were, I believe, eight hours and 24 hours. At eight hours the receptors had not been repleted. At 24 hours they were, and this was not just due to growth of new cells because in our hepatocyte system the cells do not regenerate."